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Autism Spectrum Phenotype in Males and Females with Fragile X Full Mutation and Premutation

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Abstract The behavioural phenotype of autism was assessed in individuals with full mutation and premutation fragile X syndrome (FXS) using the Autism Diagnostic Observation Scale-Generic (ADOS-G) and the Autism Diagnostic Interview (ADI-R). The participants, aged 5–80 years, comprised 33 males and 31 females with full mutation, 7 males and 43 females with premutation, and 38 non-fragile X relatives (29 males, 9 females). In the full mutation group, a total of 67% males and 23% females met either the Autism Disorder (AD) or the Autism Spectrum Disorder (ASD) criteria on at least one of the diagnostic tests. In the premutation group, 14% males and 5% females met the ADOS-G criteria for ASD. The presence of autism manifestations in males and females with full mutation and premutation provide support for a spectrum view.

Keywords Fragile X syndrome (FXS) · Fragile X premutation (FXP) · Autism Spectrum Disorder (ASD) · Autism Diagnostic Observation Schedule-Generic (ADOS-G) · Autism Diagnostic Interview-Revised (ADI-R)

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Introduction

Fragile X Syndrome (FXS) is a genetic disorder, where cognitive impairment and behavioural problems are associated with physical defects. This disorder is caused by a progressive increase in size (expansion) of a trinucleotide (CGG) repeat in the X-linked FMR1 (fragile X mental retardation 1) gene. This gene, which normally contains a repeat of 6–45 CGG trinucleotides, produces a protein (FMRP) critical for normal brain development. Expansions ranging from 55 to 200 CGG repeats, defined as 'premutation', do not cause significant FMRP deficits or obvious developmental delay (DD) (reviewed in Hagerman, 2002). If the size of the CGG repeat expands into the 'full mutation' range (> 200 repeats), this usually leads to switching off the gene, and a gross deficit of FMRP causing severe intellectual impairments (Irwin et al., 2002; Pieretti et al., 1991; Weiler, & Greenough, 1999). Fragile X females present with fewer cognitive and behavioural problems as a result of the modifying effect of a second (normal) X chromosome, with only 50–71% showing significant cognitive impairments (e.g., de Vries et al., 1996; Loesch, & Hay, 1988).

Autistic disorder (AD) is the most debilitating subgroup of a larger category known as 'Pervasive Developmental Disorders' (PDD; American Psychiatric Association, (APA) 2000) characterized by impairments in social interaction and verbal and nonverbal communication, and restricted, repetitive and

stereotypic patterns of behaviour, interests, and activities. In addition to their behavioural deficits, up to 70% of individuals with AD have an intellectual disability (Fombonne, 2003). A spectrum of autistic–like manifestations also occurs in relatives of individuals with AD. The recurrence rate in siblings is between 2% and 8%, and it is approximately 60% in monozygotic twins (Bailey, Palferman, Heavey, & Le Couteur, 1998; Cook, 2001; Le Couteur et al., 1996), indicating significant multigenic effects. Recent research suggests that up to 10 genes may be involved in the origin of AD (reviewed in Muhle, Trentacoste, & Rapin, 2004).

It is well known that the behaviuoral phenotype displayed by individuals with FXS involves features characteristic of AD, including language problems such as perseveration of speech, tactile defensiveness, poor eye contact, extreme social anxiety, and repetitive hand and finger mannerisms (e.g., Baumgardner, Reiss, Freund, & Abrams, 1995; Feinstein, & Reiss, 1998; Hagerman, 1999; Kerby, & Dawson, 1994; Lachiewicz, Spiridigliozzi, Gullion, Ransford, & Rao, 1994; Merenstein et al., 1996; Miller et al., 1999). Historical descriptions of people with FXS displaying autistic behaviours date back to the 1980s (Proops, & Web, 1981; Turner, Daniel, & Frost, 1980). Brown et al. (1982) were the first to point out this association, finding that 18.5% of males diagnosed with FXS also met the criteria for AD. Later estimates ranged from 15% to 28% (Bailey, et al., 1998; Baumgardner et al., 1995; Cohen, 1995; Hagerman, Jackson, Levitas, Rimland, & Braden, 1986; Reiss & Freund, 1990; Turk, & Graham, 1997), but a much wider range (5–60%) was encountered amongst 14 different studies (Dykens, & Volkmar, 1997), with seven reporting an incidence of more than 20%. Most of these studies utilised simple checklists of autism symtomatology and DSM diagnoses. The Childhood Autism Rating Scale (CARS, Schopler, Reichler, & Renner, 1988) was used in a sample of 57 boys with FXS (aged 2–11 years) by Bailey et al. (1998) who found that a total of 25% of boys with FXS met the CARS cutoff for autism, with most falling in the ''mild-to-moderate'' range. The discrepancy in the reported proportions may be partly attributed to the age of the participants with FXS, with autistic manifestations being more prominent earlier in life (Roger, Wehner, & Hagerman, 2001). The proportion of males with FXS who have at least some signs of autism is as high as 90% (Merenstein et al., 1996). Although autism is not a common finding in FXS females, it has been reported in both mildly and severely retarded cases (see Hagerman, 2002). Mazzocco, Kates, Baumgardner, Freund, and Reiss (1997) found that while only one of 30 such females (3%) met criteria for AD, a much larger number (17%) met criteria for a broader spectrum.

Introduction of a specific DNA test for the size of CGG repeat in the FMR1 gene has enabled accurate assessment of the proportion of individuals diagnosed as AD who are also affected with FXS. Studies have found that between 2.5% (Bailey, Phillips, & Rutter, 1996) and 6% (Brown et al., 1986; Dykens & Volkmar, 1997) of males with a diagnosis of AD have FXS. Bailey et al. (1993) also found that 5% of 40 girls with a diagnosis of autism screened positive for FXS. The average estimate of the proportion of FXS positive individuals, based on review of several studies, was 6.5% in males and 4% in females with autism (Bailey et al., 1993; Hagerman, 1991).

Early research charting the relationship between FXS and autism was hampered by using different diagnostic instruments, such as DSM checklists or the CARS, which rely on limited information. The CARS, in particular, has been designed for assessing autism in young children, and is therefore not suitable for use across a wider age range. Furthermore, it has been acknowledged that this test may indicate autism in children with severe delays because of the delay itself (Bailey, Hatton, Mesibov, Ament, & Skinner, 2000).

An important development in both the clinical and research realms has been the introduction, and almost universal acceptance, of two diagnostic tools for autism: the Autism Diagnostic Interview (ADI-R, Lord, Rutter, & Le Couteur, 1994) and the Autism Diagnostic Observation Scale- Generic (ADOS-G, Lord, Rutter, DiLavore, & Risi, 1999), which have been validated across different ages and severity levels. The ADI-R is a semi-structured, standardised parent interview that assesses the presence and severity of early childhood symptoms of autism across the three main domains: impairments in reciprocal social interaction; impairments in communication; and restricted, repetitive and stereotyped patterns of behaviour. The ADI-R employs an algorithmic scheme, combining scores for those items found to be most discriminating of autism, providing an overall classification of AD based on reaching cutoffs for these three domains.

The ADOS-G is a semi-structured standardised assessment administered directly to the affected individual, and it complements the ADI-R in classifying AD. The ADOS-G uses developmentally appropriate social and play-based interactions as well as interview questions designed to elicit spontaneous behaviours across the following areas: reciprocal social interaction; language and communication; play and imagination; and stereotyped, repetitive and restrictive behaviours (Lord et al., 1999). The ADOS-G is unique in that it

consists of four different modules, which are selected based on chronological age and language ability. One of the important features of the ADOS-G algorithm is that it discriminates between the narrower definition of AD and the broader definition of Autism Spectrum Disorder (ASD), which is a milder form of AD. The ASD diagnosis is appropriate when thresholds are generally lower than the cutoff for AD, or when not all cutoffs are met.

The only earlier study, to our knowledge, to use both the ADI-R and the ADOS-G in individuals with FXS was that by Rogers et al. (2001). They administered the ADI-R and the Module 1 ADOS-G to 3 groups of 2–4 year-old children (predominantly boys): an AD group, a developmentally delayed group, and an FXS group. The 33% of FXS individuals who met the cutoffs for AD on both the ADOS-G and the ADI-R had very similar behavioural profiles to the AD group. The remaining FXS children, who did not meet the cutoffs for AD, presented with a behavioural profile that was almost identical to the group with DD. Rogers et al,. (2001) also found that the young boys with FXS and AD had both lower developmental and adaptive functioning scores than boys with FXS only, confirming other findings of lower levels of functioning in individuals with both these conditions (Bailey, Mesibov et al., 1998; Bailey et al., 2000).

The aim in the present study was to estimate the prevalence of autism in a sample of both males and females affected with full mutation (FXS), or carrying a premutation in the FMR1 gene, using both the ADI-R and the ADOS-G. In so doing, we included individuals within a wide age range, as we were interested in investigating the relationship of this prevalence with chronological age as well as cognitive abilities and adaptive functioning.

Method

Participants

A total of 152 participants aged 5–80 years were included in this study. Sixty-four individuals (33 males, 31 females) were affected with full mutation, 50 were premutation carriers (7 males, 43 females), and 38 were non-fragile X relatives (29 males, 9 females). Of the 33 full mutation males, 23 were probands and of the 31 full mutation females, 6 were probands. Two of the 7 premutation males and 4 of the 43 premutation females were probands. Participants in the full mutation group were aged between 5.75 and 60.67 years (mean: 23.15), those in the premutation group were between 5.42 and 79.67 years (mean: 35.70), and the non-FXS relatives were aged 6.25–63.67 years (mean: 31.46).

All aspects of this study were approved by the ethics committees of La Trobe University and the Royal Children's Hospital in Melbourne, and by the Institutional Review Board of the University of California at Davis. Participants and/or their parents gave written informed consent. The Caucasian subjects, all Australian residents, were recruited, in an unbiased manner, from the La Trobe register of families participating in our other on-going NIH and/or NHMRC supported Fragile X studies. These families were initially ascertained through clinical admissions of probands diagnosed with FXS at the Victorian Genetic Health Services at the Royal Children's Hospital in Melbourne, and then cascade-tested by the investigators (DZL & AKT). Individuals with serious associated medical conditions were not included, but those with corrected vision and hearing impairments or minor medical problems were tested.

Fragile X status was established using a specific DNA test performed at Kimball Genetics, Inc. (Denver, CO), as detailed in Taylor et al. (1994), and Loesch et al. (2002). The DNA results were used here as a diagnostic tool in order to classify individuals into premutation, full mutation, and non-fragile X, status categories. Because of low numbers, a single grey-zone individual has been included in the normal category, and premutation/full mutation mosaics, as well as unmethylated full mutations (approximately 20% of the affected males), have been included in the full mutation category.

Autism Measures

Social-Communication Questionnaire (SCQ)

The 40-item Social-Communication Questionnaire (SCQ) asks parents about their child's social and interactive behaviour (Rutter et al., 2001). It was used here as a screening test, with a cut-off score of 15 or over indicating an increased risk of autism. Both the ADI-R interviews and ADOS-G tests were conducted only in those participants who were above the cut-off SCQ score, irrespective of their fragile X status.

ADI-R

This interview elicits complete descriptions of current and early autistic behaviour in response to 92 questions. A code from 0–3 represents the severity of the specified behaviour. The coding algorithm specifies a

threshold score of 8 on the communication scale for verbal subjects and seven for non-verbal subjects. The threshold score is 10 on the social scale, and three on the restricted stereotyped and repetitive behaviours. In addition, to meet the classification criteria of AD, the participant must have exhibited some abnormality in the first 36 months of life as described by the caregiver and judged by the interviewer.

ADOS-G

This tool has four Modules, with Modules 1–3 used in children with increasing verbal fluency, and Module four is used for verbally fluent adolescents and adults as it focuses on social-emotional questions and daily living skills. The ADOS-G items are rated from 0 to 3 depending on the severity of typical specified behaviour.[1] The ADOS-G also adopts an algorithmic scheme, with scores for the items greatest discriminating power being combined into summary scores for four the categories: (1) language and communication; (2) reciprocal social interaction; (3) play and imagination for M1 and imagination and creativity for Ms 2–4; and (4) stereotyped, repetitive and restrictive behaviours (Lord et al., 1999).

In order to meet the criteria for AD, an individual must reach or exceed the following thresholds on each of the three categories: communication (cutoffs: M1 and M2 = 5; M3 and M4 = 3), reciprocal social interaction (cutoffs: M1 = 7; M2 = 6; M3 and M4 = 6), and social-communication total (cutoffs: M1 = 12 and M2 = 12; M3 and M4 = 10). The algorithm discriminates between the narrower definitions of AD and the broader definitions of ASD, based on severity, with the latter being used when thresholds are generally lower than the cutoff for autism, or not all three are met. For a classification of ASD, the thresholds are as follows: Communication (COMM; cutoffs: M1 = 2; M2 = 3; M 3 and M4 = 2), Reciprocal Social Interaction (RSI; cutoffs: M1, M2, M3 and M4 = 4), and Social-Communication Total (CSIT; cutoffs: M1 = 7; M2 = 8, M3 and M4 = 7).

Cognitive and Adaptive Behaviour Measures

Wechsler Intelligence Scales (WISC) were used to assess general intellectual functioning (FSIQ). Individuals under the age of six were tested using the WPPSI-R and the WPPSI-III (Wechsler, 2002), participants aged between 6 and 16 were tested using the WISC-III (Wechsler, 1991), and individuals over the age of 16 were tested using the WAIS-III (Wechsler, 1997).

The Vineland Adaptive Behaviour Scales Interview Edition (VABS)

This parent interview assesses current adaptive behaviour in both personal and social areas (Sparrow, Balla, & Cicchetti, 1984). It consists of three domains: Communication (COMM), Daily Living Skills (DLS), and Socialization (SOC). The Adaptive Behaviour Composite (ABC) is a total score derived from these domains. Each subscale includes both standard scores and developmental equivalents; standardized scores were used throughout this study.

Procedure

Cognitive testing was conducted in the participants' homes with all except three individuals who were assessed in a clinical setting. Breaks were given, and if any of the measures were not completed on the day, a later collection time was arranged. Administration of the ADI-R and ADOS-G required extensive training before administration commenced. The primary rater in this study (SC) who administered these tests was trained at Monash University by a member (Dr. Christina Corsello) of Dr. Catherine Lord's team (University of Chicago) to a reliability of 85% or better item agreement on the full range of scores (0–3). Participants' mothers were administered the VABS during a structured interview.

Results

Autism Classification

Only one out of the 38 (2.6%) non-fragile X relatives exceeded the threshold score on the SCQ (SCQ+). One out of 7 (14.3%) premutation male carriers, and 3 out of 43 (7%) premutation female carriers exceeded the threshold score of 15 on this screening measure. Amongst the individuals with full mutation, 23 males out of 33 (69.7%) and 7 females out of 31 (22.6%) exceeded the threshold score of 15 on the SCQ.

The numbers of the SCQ+ premutation and full mutation males and females meeting the AD criteria on the ADI-R and/or ADOS-G, and ASD criteria on the ADOS-G, are presented in Table 1. Percentage frequencies relative to the total number of individuals

[1] The rating of seven is given when abnormal behaviour of a type that is not encompassed by the other ratings is apparent, and eight is assigned when the behaviour does not occur and/or the rating is not applicable. These latter ratings cannot be used in the algorithm and are therefore given sparingly.

Table 1 The number (and %) of premutation and full mutation individuals meeting criteria for autistic disorder (AD) on the ADI-R and the ADOS-G, and autism spectrum disorder (ASD) on the ADOS-G

		Males		Females		Total	
		n	%	*n*	%	*N*	%
Premutation		(*n* = 7)		(*n* = 43)		(*n* = 50)	
AD	ADI-R	0	–	1	2.3	1	2.0
	ADOS	0	–	0	–	0	–
	Both	0	–	0	–	0	–
	Total	0	–	1	2.3	1	2.0
ASD	ADOS	1	14.3	2	4.7	3	6.0
Full mutation		(*n* = 33)		(*n* = 31)		(*n* = 64)	
AD	ADI-R	6	18.2	1	3.2	7	10.9
	ADOS	6	18.2	1	3.2	7	10.9
	Both	6	18.2	3	9.7	9	14.1
	Total	18	54.5	5	16.1	23	35.9
ASD	ADOS	7	21.2	3	9.7	10	15.6

in each group, irrespective of the SCQ screening outcome, are also given.

None of the premutation males and females met autism criteria (AD or ASD) on both diagnostic instruments. However, one female (2.3%) met AD criteria on the ADI-R, and one (14.3%) male and two females (4.7%) met the ASD criteria on the ADOS-G; none met the criteria for AD on the ADOS-G.

In the full mutation group, 6 males (18.2%) met the criteria for AD on the ADI-R and 6 met the AD criteria on the ADOS-G. A further 6 (18.2%) full mutation males met these (stringent) AD criteria on both the tests. Three of the 6 males who met AD criteria on the ADI-R also met the less stringent ASD criteria on the ADOS-G. Thus a total of 9 (27%) full mutation males met autism (AD or ASD) criteria on both tests. If a broader autism spectrum is considered, a total of 22 males (67%) were classified as either AD (on one of the tests) or ASD (on the ADOS-G).

One (3.2%) full mutation female met the criteria for AD on the ADI-R and another one (3.2%) met these criteria on the ADOS-G. A further 3 (9.7%) full mutation females met AD criteria on both the tests. The female who met AD criteria on the ADI-R only also met criteria for ASD on the ADOS-G. Thus all four full mutation females who met the criteria for ADI-R also met one of the criteria on the ADOS-G, giving a total of 13% of females who met autism criteria (AD or ASD) on both tests. If we consider the number of females who met at least one of the criteria on one of the tests, a total of 7 (23%) females were classified as either AD (on one of the tests) or ASD (on the ADOS-G).

Two premutation males and four premutation females were probands. Amongst the full mutation individuals, 23 males and six females were probands. Fig. 1 illustrates the percentage of full mutation and premutation individuals classified as either AD or ASD (on one of the tests) who were either probands or non-probands. Eighteen of the 23 full mutation male probands and four of the six full mutation female probands were classified as AD/ASD. In the premutation sample, one of the two male probands and one of the four female probands were classified as AD/ASD. As Fig. 1 illustrates, a higher percentage of probands across both fragile X categories and genders were classified as having autism.

Autism Behaviour Profiles of Full Mutation Individuals Meeting ADOS-G criteria

The profile of mean scores on the ADOS-G and ADI-R domains in the sample of 16 full mutation male and female participants who met the criteria for AD, and the sample of 10 full mutation male and female participants who met the ASD criteria on the ADOS-G, are presented in Fig. 2a, b, respectively. Predictably, the data in Fig. 2a show higher mean scores for both COM and RSI domains, and for the total CSIT, in

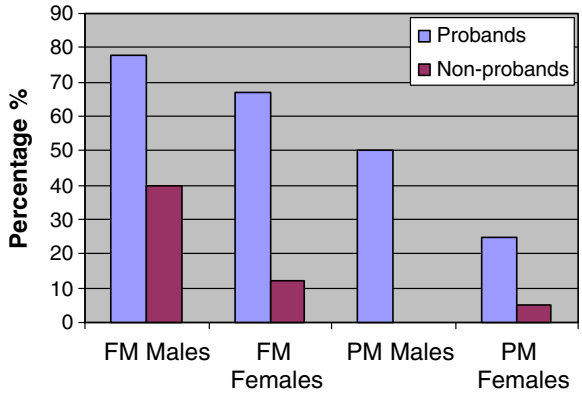

Fig. 1 Percentage of probands and non-probands in each of the full mutation (FM) and premutation (PM) groups who were classified with AD/ASD

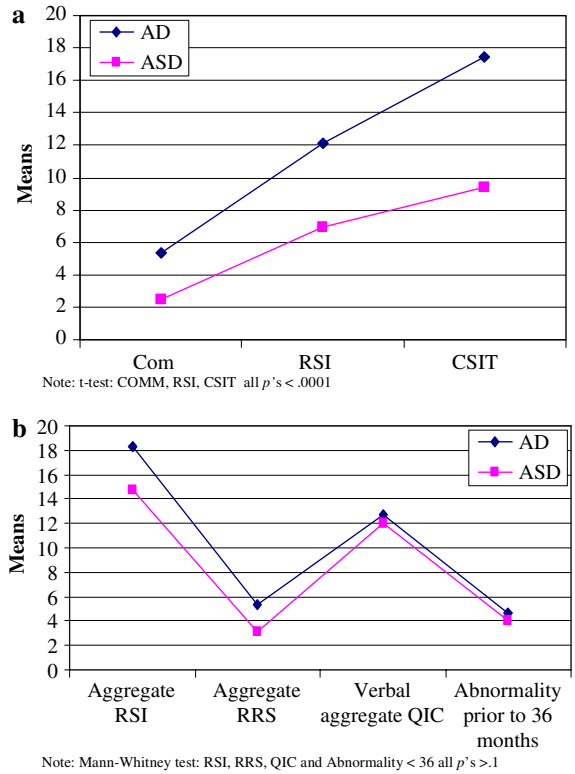

Fig. 2 (**a**) ADOS-G profiles and (**b**) ADI-R profiles for AD (n = 16) and ASD groups (n = 10) full mutation subgroups

individuals meeting the criteria for AD on the ADOS-G, than those meeting criteria for ASD, with significant differences between these groups. In contrast, the mean scores for the ADI-R domains in these same groups is almost identical (Fig. 2b).

Comparisons Between the Full Mutation Individuals With and Without Autism

The full mutation individuals meeting the AD or ASD criteria on the ADOS-G (the FXS+ASD group, males and females combined, n = 26) were compared to those not meeting these criteria (the FXS ONLY group, n = 38) on chronological age, cognitive ability and adaptive functioning. The AD and ASD thresholds from the ADOS-G were chosen in order to maximize group numbers. The results in Table 2 show significant group differences, with the FXS+ASD group scoring lower on cognitive and adaptive functioning. Moreover, the FXS+ASD individuals were significantly younger than their FXS ONLY counterparts. This difference may be attributed to the effect of 'anticipation' typical of disorders caused by unstable mutation, where the younger generations are more severely cognitively and behaviourally affected than the older ones due to progressive increase in the (CGG) repeat sise. In order to establish if this bias entirely accounts for the observed differences in the level of cognitive impairment between FXS+ASD and FXS ONLY samples, we compared these groups using ANCOVA, with age included as a covariate. The results in Table 2 show that the differences between groups remain significant for all measures included. However, upon covarying FSIQ, the differences between the FXS+ASD and FXS only groups on adaptive behaviour are no longer significant (see Table 2).

Discussion

The first study aim was to establish the prevalence of autism (AD and ASD) amongst males and females of a wide age range affected with FXS, or carrying premutation in a fragile X gene, using current gold standard tools, the ADI-R and ADOS-G. Eighteen percent of FXS males met the stringent criteria for AD on both tests. This rate is considerably lower than the prevalence of 33% meeting AD criteria found by Rogers et al. (2001) in their sample of 24 children with FXS, using the same diagnostic tools. This may be due to the fact that our sample comprised adolescents and adults, as well as children, compared with Rogers et al. who only studied young children between the ages of 21–48 months. Bailey, Mesibov et al. (1998) also found a higher percentage (25%) of boys with FXS meeting autism criteria, although direct comparisons are hard to make given their reliance on the CARS. However, if we consider a more ''relaxed'' approach to estimating prevalence, 27% of full mutation males in our study met the cutoffs on both the ADOS-G (using ASD and AD criteria) and the ADI-R. Taking an even broader spectrum view, we found that that 67% of males with full mutation meet at least of one of the AD/ASD criteria on one test, providing support to the claim that autistic behaviour is a major component of the fragile X phenotype (Bailey, Hatton, Skinner, & Mesibov, 2001; Hagerman, 1999).

This is the first study on the prevalence of autistic behaviour in full mutation FXS females using the two gold standard diagnostic tools. Taking a stringent approach to prevalence estimation, 9.7% of FXS females met the AD criteria on both tests, nearly half the rate seen in males, which should be expected for an X-linked disorder. A somewhat higher prevalence (13%) was found when using both the ASD/AD criteria on the ADOS-G and the AD criteria on the ADI-R. Moreover, like in males, the frequency is considerably higher (23%) when considering the broader spectrum (AD or ASD) on one of the tests, again indicating the high prevalence of autism manifestations in FXS.

Table 2 Summary statistics and *p*-values (*p*) for differences between the ASD and Non-ASD full mutation groups for chronological age, FSIQ and the vineland adaptive behaviour domains (COMM, DLS, SOC) and total vineland score (ABC)

	ASD			Non-ASD			Unadjusted	Adjusted for age	Age covariance	Adjusted for FSIQ	FSIQ covar.
	n	Mean	*SD*	*n*	Mean	*SD*	*P*	*P*	*P (age)*	*P*	*p (FSIQ)*
Age	26	18.21	10.77	38	26.53	13.43	0.0094[a]				
FSIQ**	21	48.00	5.78	38	69.31	17.65	< 0.0001[a]	< 0.0001	0.1208		
COMM	24	32.54	15.18	24	56.13	28.36	0.0008[b]	< 0.0001	0.0004	0.2294	0.0007
DLS	24	35.46	22.36	24	64.75	28.46	0.0003[b]	0.0005	0.6416	0.3186	< 0.0001
SOC	24	46.00	23.25	24	62.00	27.33	0.0341[b]	0.0113	0.0512	0.6926	< 0.0001
ABC*	24	36.54	17.94	24	55.96	25.10	0.0029[a]	0.0006	0.0418	0.5475	0.0001

* Two-side *p*-value calculated based on two-sample

** *p*-value for age/FSIQ adjusted base on logarithm transform data

[a] Mann-Whitney and [b] t-test statistics

The prevalence figures become more meaningful when they are considered separately for probands and non-probands identified through cascade testing. Since the major abnormality in FXS is intellectual impairment, the probands are selected through this particular trait, so that autistic behaviour should not, in principle, be directly relevant to ascertainment (Thompson, 1993). Contrary to this assumption, however, we have found a large difference in the prevalence of autism between probands and non-probands. This may be explained, at least for the male sample, by a strong association between autism and intellectual impairment, as found in the present data and in earlier studies (Bailey, Mesibov et al., 1998; Bailey et al., 2000; Kaufmann et al., 2004; Loesch et al., submitted; Rogers et al., 2001). However, our results for females, where the overwhelming majority of FXS+ASD individuals came from the sample of probands, suggest that behavioural problems, rather than cognitive impairment, may be more relevant to ascertainment. These two postulated sources of ascertainment have implications for the way future fragile X studies correct for an ascertainment bias.

In addition to providing prevalence estimates of autism behaviours in fragile X males and females, our data support the concept of a spectrum of autism manifestations in FXS, rather than a dichotomous classification into autism as opposed to non-autism categories, as suggested by Rogers et al. (2001). The concept of a spectrum is reinforced by our finding that the behavioural profiles of individuals meeting the ASD criteria and the AD criteria on the ADOS-G were very similar on both the ADOS-G and the ADI-R domains. The evidence for a spectrum of autism manifestations, rather than a distinct category of autism, is even stronger considering that, in our study, 14.3% of premutation males and 4.7% of premutation females met the ASD criteria, which corresponds to the lower end of the distribution for the whole spectrum of autistic manifestations. In agreement with our view, Kaufmann et al. (2004) further extended the spectrum notion by identifying a broader range in FXS with respect to autistic manifestations, including, FXS+AD, FXS+ASD, and FXS + PDD.

The data, which suggests a spectrum of autism manifestations related to the spectrum of cognitive impairment, argues against the hypothesis that autism in FXS originates from some additional predisposing genetic influences, where fewer background 'autism' genes are sufficient to generate an autism phenotype in individuals already affected by FXS (Le Couteur et al., 1996; Bailey et al., 2001; Rogers et al., 2001). Instead, the present data, as well as our earlier findings of a strong association between FSIQ and autism based on a quantitative approach (Loesch et al., submitted), and the fact of widespread occurrence of autism in a number of chromosomal (Gillberg, 1998; Fombonne, Du Mazaubrun, Cans, & Grandjean, 1997; Muhle et al., 2004) and genetic (Dykens & Volkmar, 1997; Fombonne et al., 1997; Muhle et al., 2004) syndromes associated with developmental delay, indicate that cognitive impairment may solely account for the co-morbidity between FXS and autism. This thesis was suggested more than a decade ago (Fisch, 1993).

The somewhat unexpected finding of a close similarity between FXS individuals with AD and ASD on the ADI-R domains may be related to an age effect on the level of autism manifestations. The individuals classified as having ASD later in life could have been more severely affected at the younger age, on which the ADI-R is based. Indeed, it has been shown previously (Bailey, Mesibov et al., 1998; Rogers et al., 2001) that the severity of autistic manifestations is greater in younger children, especially those between 4 and 5 years of age. This finding can be interpreted as the result of between generation differences in

ascertainment due to anticipation, which result, as was first shown by Loesch, Sheffield, & Hay (1993), in the younger generations comprising more severely affected individuals carrying larger expansions and being more severely intellectually impaired than the older generations. Since there is evidence for a relationship between cognition (FSIQ) and autism manifestations (e.g., Bailey, Mesibov et al., 1998; Bailey et al., 2000; Rogers et al., 2001; Loesch et al., submitted), we claim that the observed inverse relationship of autism scores with age is the result of between generation differences in severity of cognitive deficit and autistic manifestations.

The slight discrepancies in the classification of individuals as AD using the ADOS-G and the ADI-R tests deserves special comment. The interaction between the true age- dependency of autism manifestations, with young children being more severely affected, and reliability of the informant, most probably contributes to this discrepancy. That is, although the full syndrome of autism in individuals with FXS is more prominent earlier in life on which the ADI-R is focused, this test is also based on caregiver report of behaviours, often occurring many years earlier. Because our sample comprised individuals of a wide age range, being, on average, much older than those in the previous studies using the ADI-R (Kaufmann et al., 2004; Rogers et al., 2001), mothers may have had significant problems in remembering the critical 4–5 year range on which the ADI-R is based. Additionally, because the mothers of fragile X individuals are themselves carriers of premutation or full mutation, their emotional problems and/or cognitive deficits (see Hagerman, 2002) may have interfered with accurate and objective reporting of their child's early development. Indeed, the group of mothers interviewed in our study had a mean IQ of 89 (SD = 25.1). The ADOS-G, on the other hand, relies on direct observation of the individual by a trained expert, allowing subtle distinctions to be made in a controlled environment, and the majority of our participants were adolescents and adults when they were observed.

In conclusion, the study findings suggest that further investigations of the associations between autistic behaviours and cognitive impairments in large samples of individuals with FXS, as well as in individuals with other genetic or chromosomal mental retardation syndromes, are important for understanding the neurobiology of autism, on the one hand, and the mechanisms leading to severe behavioural problems in fragile X, on the other.

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